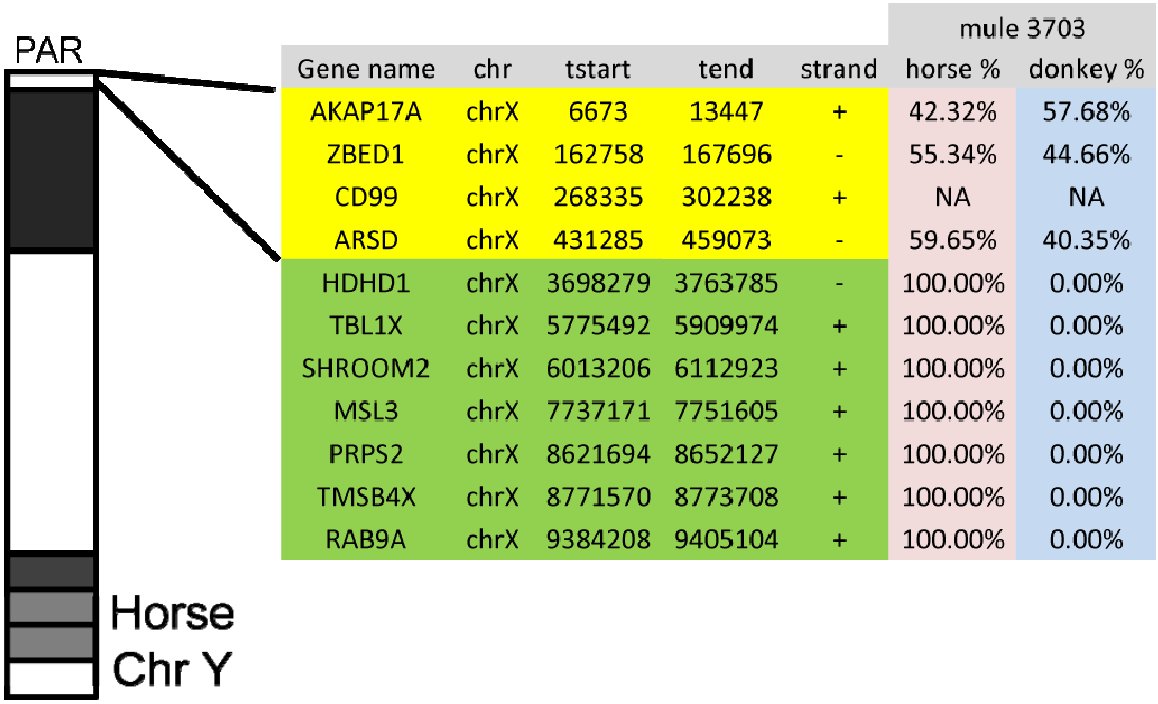
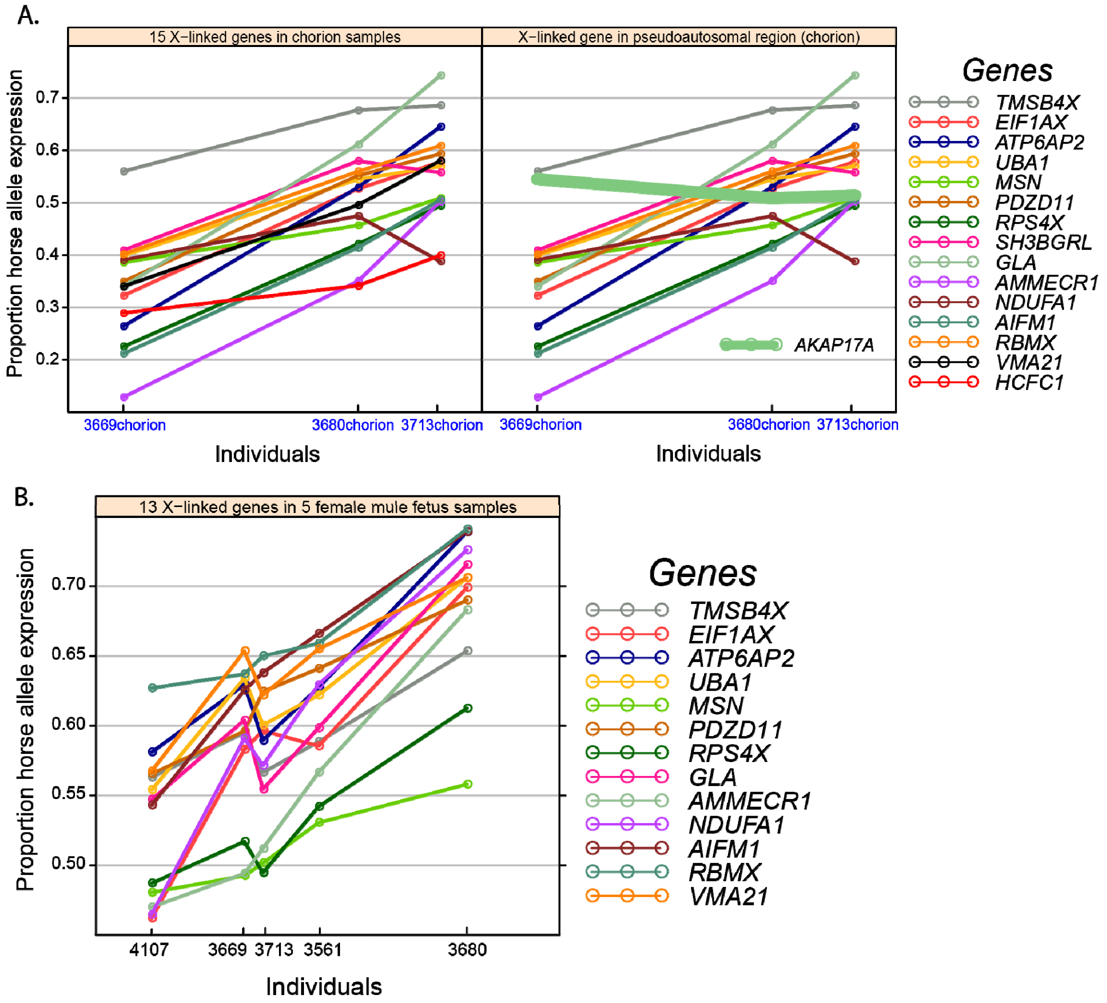
Supplemental Figure S1. Allelic expression percentages in male mule3703 across the PAR boundary.



					mule 3703	
Gene name	chr	tstart	tend	strand	horse %	donkey %
AKAP17A	chrX	6673	13447	+	42.32%	57.68%
ZBED1	chrX	162758	167696	-	55.34%	44.66%
CD99	chrX	268335	302238	+	NA	NA
ARSD	chrX	431285	459073	-	59.65%	40.35%
HDHD1	chrX	3698279	3763785	-	100.00%	0.00%
TBL1X	chrX	5775492	5909974	+	100.00%	0.00%
SHROOM2	chrX	6013206	6112923	+	100.00%	0.00%
MSL3	chrX	7737171	7751605	+	100.00%	0.00%
PRPS2	chrX	8621694	8652127	+	100.00%	0.00%
TMSB4X	chrX	8771570	8773708	+	100.00%	0.00%
RAB9A	chrX	9384208	9405104	+	100.00%	0.00%

In this figure, the horse and donkey allelic expression percentages are shown for transcribed X-linked genes in mule-3703 (male). Biallelic expression from both parental alleles was observed for genes in the pseudo-autosomal region (*AKAP17A*, *ZBED1*, *CD99* and *ARSD*), which have one copy on the X chromosome and one copy on the Y chromosome, resulting the 1-3% overall paternal expression (Fig. 2a). Across the PAR boundary, X-linked genes outside PAR show 100% maternal expression from the horse allele.

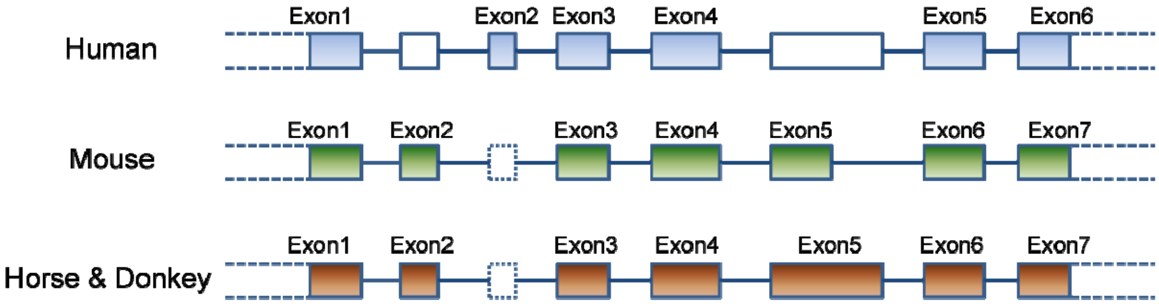
Supplemental Figure S2. Relative allelic expression levels in chorion and fetal samples based on pyrosequencing verification data.



(A). Plot of allelic expression proportions in three female mule chorion samples for 16 X-linked genes based on pyrosequencing data. In the left panel are the allelic expression profiles of 15 selected X-linked genes in three female mule chorion samples from pyrosequencing verifications. The *x*-axis simply arrays the three individuals in rank order by mean relative expression. Plotted on the *y*-axis is the proportion of expression from horse allele. The PAR gene *AKAP17A* is highlighted in the right panel. The 15 genes are the same as the genes tested in chorionic girdle samples.

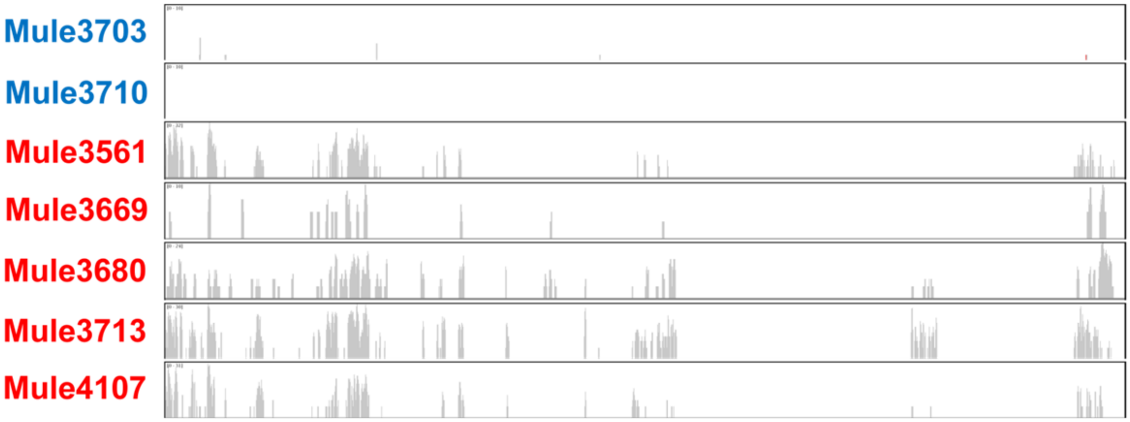
(B). Plot of allelic expression proportions in fetal samples of five female mules for 13 X-linked genes from pyrosequencing verification data. Plotted in this figure are the allelic expression profiles of 13 selected X-linked genes in five female mule fetus samples from pyrosequencing verification. The *x*-axis presents the five mule individuals with individual ID labeled ordered by mean relative expression rank. Plotted on the *y*-axis is the proportion of expression from horse allele.

Supplemental Figure S3. Annotation of the horse and donkey XIST gene model and comparison with human and mouse.



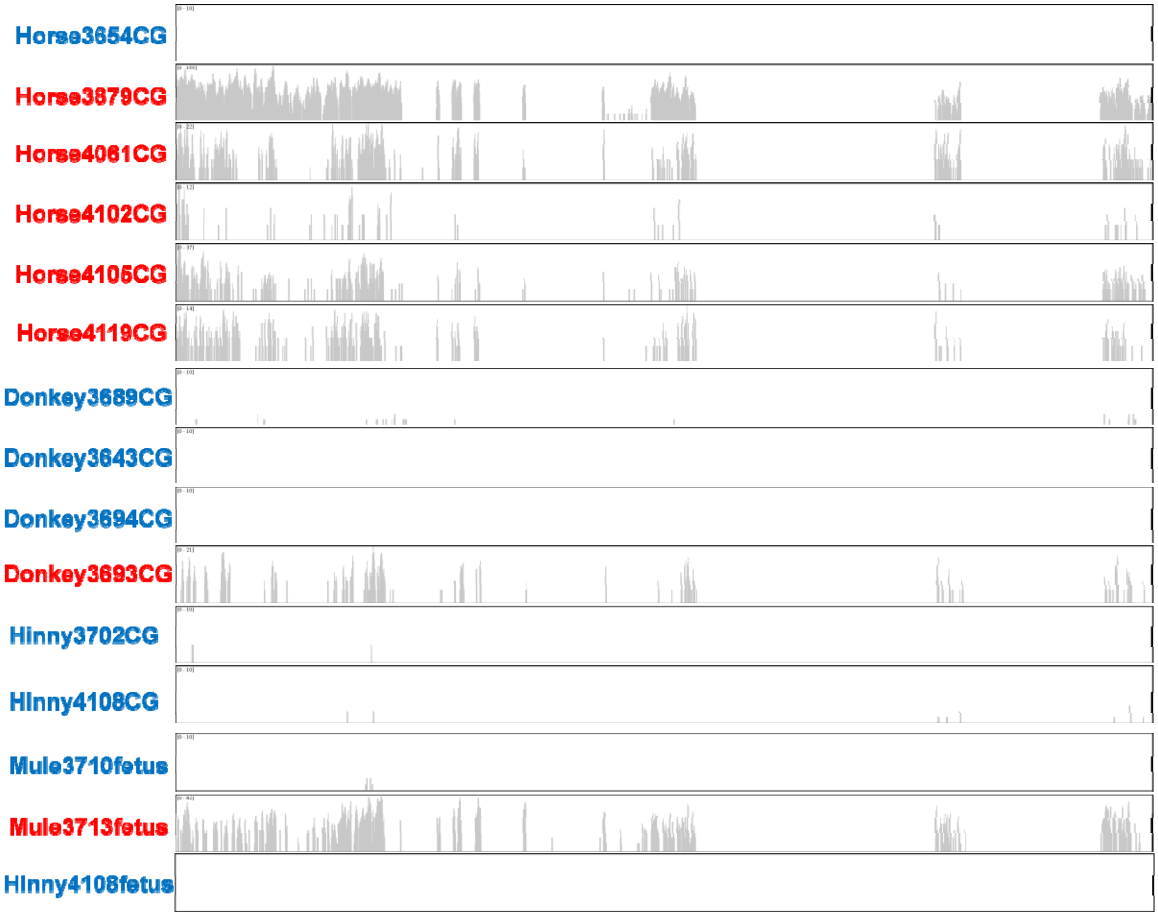
Human *XIST* gene model (in blue) was drawn based on the RefSeq gene model NR_001564. The six human exons in the RefSeq gene model are all expressed. Human has the two exons which are orthologous to mouse *Xist* exon 2 and 6, but they are expressed in human *XIST* transcript (indicated by uncolored boxes). The mouse *Xist* gene model (in green) was based on the RefSeq gene model NR_001463. The horse/donkey *XIST* gene model (in brown) was annotated from our female horse, donkey and mule RNA-seq data. There are seven expressed exons in the mouse and horse *XIST* genes. The human exon 2 is absent in the mouse and horse (boxes with dash lines).

Supplemental Figure S4. RNA-seq coverage of XIST transcript in seven mule chorionic girdle samples, showing the expression profile.

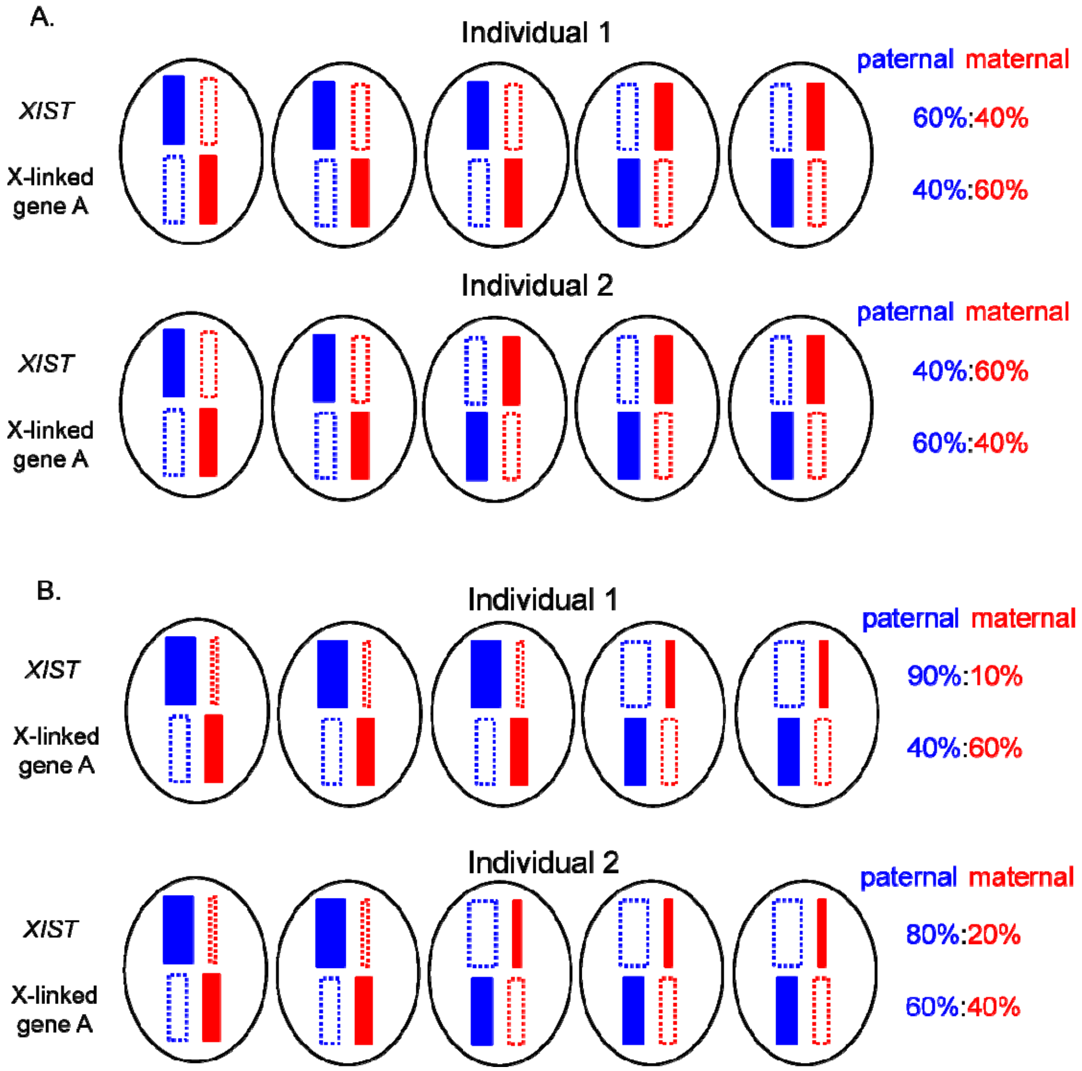


The chorionic girdle sample RNA-seq read coverage for the 27 kbp *XIST* gene region (chrX:55,226,251-55,258,045bp) was plotted for two male (labeled in blue) and five female (labeled in red) mules.

Supplemental Figure S5. RNA-seq coverage of *XIST* transcript (27 kbp gene region) in horse donkey and hinny chorionic girdle and mule and hinny fetus samples, showing the expression profile. Males were labeled in blue and females in red.



Supplemental Figure S6. Scheme of the allelic expression of *XIST* and X-linked genes that are subject to X inactivation.



Tissues from two individuals with five cells are drawn in each figure. In each cell, the *XIST* expression status is on the top and the expression status of other X linked genes is on the bottom. Paternal expression is shown in blue and maternal expression in red. A dotted box means no expression. The width of the box is in proportion to the relative expression level.

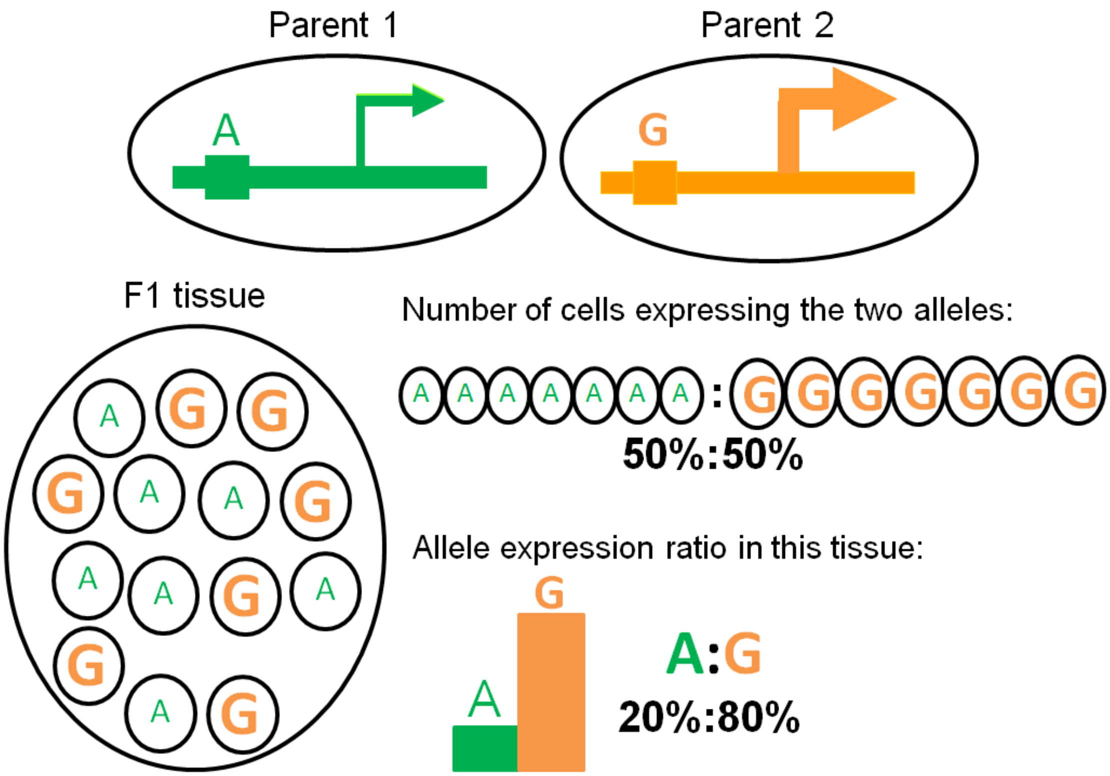
(A) Similar *Xce* allele strength of the two parental X chromosomes.

In each cell of this diagram, *XIST* is monoallelically expressed from the inactive X chromosome, and its expression level from the maternal and paternal allele is similar. Individual 1 has three cells out of five expressing the paternal *XIST*, therefore 60% maternal expression is observed for other X-linked genes that are subject to random X inactivation. Individual 1 has two cells expressing the paternal *XIST*, and 40% maternal expression is observed. The allelic expression ratios for X-linked genes can be predicted by the *XIST* allelic expression ratio, which is the situation we observed in mule.

(B) Strong *Xce* effect favoring expression of the paternal *XIST*.

In each cell of this diagram, *XIST* is monoallelically expressed from the inactive X chromosome, but the paternal expression level is much higher due to the *Xce* effect. Although the X-linked gene expression ratios for individual 1 and 2 are the same as in (A), we always observe higher expression level from the paternal *XIST* allele.

Supplemental Figure S7. Diagram of *cis*-eQTL effect in F1 hybrids.



In this Figure, the *cis*-eQTL effect is demonstrated in F1 hybrids when there is monoallelic expression in each cell under random X inactivation. QTL is a polymorphic locus underlying a quantitative trait, with alleles that differentially affect this continuous phenotypic trait. In *cis*-eQTL (*cis*-regulating expression quantitative trait locus), the trait is allelic expression in *cis*. In the top panel of this figure, there is a *cis*-regulating polymorphism at the promoter region of an X-linked gene between Parent 1 (A allele) and Parent 2 (G allele). The G allele has 4-fold higher expression compared to the A allele. In a specific tissue of the F1 animal (bottom left panel), due to random X inactivation, 50% of the cells are expressing the A allele and 50% cells are expressing the G allele. However, if the A/G allelic expression is quantified in this tissue as a whole, 20% A allele and 80% G allele expression is observed as a result of the *cis*-eQTL effect. *cis*-eQTL is a strains-specific effect and can vary among different genes.

Supplemental Figure S8. Correlation of average allelic expression propotion across 12 X-linked genes in five female mules between fetus and chorionic girdle.